

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

Date of mailing:

15 March 2001 (15.03.01)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

International application No.:

PCT/EP00/08587

Applicant's or agent's file reference:

5213/00/WO-Por

International filing date:

02 September 2000 (02.09.00)

Priority date:

04 September 1999 (04.09.99)

Applicant:

SCHNELL, Urban et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International preliminary Examining Authority on:

22 December 2000 (22.12.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 5213/00/WO-Por	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/08587	International filing date (<i>day/month/year</i>) 02/09/2000	(Earliest) Priority Date (<i>day/month/year</i>) 04/09/1999
Applicant F.HOFFMANN-LA ROCHE & CO		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. **Certain claims were found unsearchable** (See Box I).

3. **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

2

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/08587

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 B01L7/00 //G01N21/64, G01N21/05, C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHEDMinimum documentation searched (classification system followed by classification symbols)
 IPC 7 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 38487 A (POURAHMADI FARZAD ;CEPHEID (US); YUAN ROBERT (US); CHANG RONALD (U) 3 September 1998 (1998-09-03) page 3, line 27 -page 5, line 2 page 6, line 20 -page 8, line 6 page 12, line 4 -page 14, line 6 page 14, line 1 -page 15, line 21 page 16, line 25 -page 18, line 13 page 20, line 5 -page 20, line 8 page 21, line 24 -page 22, line 26 figures 1-6 ----	1-10, 14-21, 25,26
Y	----- -/-	1,4-8,14

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 December 2000	13/12/2000

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
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Authorized officer

Koch, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/08587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 318 255 A (EASTMAN KODAK CO) 31 May 1989 (1989-05-31) column 3, line 11 -column 4, line 14 column 6, line 45 -column 7, line 20 column 7, line 56 -column 9, line 20 column 10, line 13 -column 11, line 4 column 13, line 28 -column 13, line 46 column 14, line 27 -column 15, line 32 figures 1-21 ----	14,21,22
A	column 11, line 19 -column 11, line 51	17
Y	column 13, line 28 -column 13, line 46 column 14, line 27 -column 15, line 32 figures 1-21 ----	12,23
P,Y	US 6 087 182 A (TSYY-WEN JENG ET AL) 11 July 2000 (2000-07-11) column 11, line 19 -column 11, line 51 column 14, line 48 -column 15, line 11 column 16, line 12 -column 16, line 36 column 16, line 50 -column 18, line 28 figures 1-9 ----	1,4-8,14
Y	US 5 856 174 A (FODOR STEPHEN P A ET AL) 5 January 1999 (1999-01-05) & US 6 043 080 A (FODOR STEPHEN PA ET AL) 28 March 2000 (2000-03-28) column 2, line 14 -column 3, line 8 column 6, line 19 -column 6, line 44 column 8, line 40 -column 8, line 54 column 13, line 21 -column 13, line 41 column 14, line 7 -column 14, line 40 column 15, line 65 -column 16, line 36 column 17, line 22 -column 19, line 24 column 19, line 54 -column 20, line 9 column 22, line 64 -column 24, line 46 figures 1-10 ----	12,23
X	WO 96 41864 A (UNIV CALIFORNIA) 27 December 1996 (1996-12-27)	1,2,4, 14,18, 25,26
A	page 3, line 26 -page 5, line 13 page 7, line 17 -page 8, line 23 figures 1,2 ----	1,4,5
A	US 5 567 617 A (CAPRIO CRAIG A ET AL) 22 October 1996 (1996-10-22) cited in the application -& EP 0 662 345 A (CLINICAL DIAGNOSTICS SYSTEMS INC) 12 July 1995 (1995-07-12) column 3, line 15 -column 4, line 34 column 5, line 35 -column 5, line 39 column 7, line 38 -column 8, line 45 figures 1-10 ----	1-3,5, 10,14, 15,19, 20,25,26
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/08587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 580 362 A (TOSOH CORP) 26 January 1994 (1994-01-26) page 2, line 53 -page 3, line 12 page 3, line 30 -page 3, line 32 page 4, line 47 -page 5, line 20 figures 1,2 -----	1,4-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/08587

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9838487	A 03-09-1998	US 5958349 A			28-09-1999
		AU 6343398 A			18-09-1998
		EP 0963545 A			15-12-1999
EP 0318255	A 31-05-1989	US 4902624 A			20-02-1990
		DE 3876957 A			04-02-1993
		DE 3876957 T			08-07-1993
		DE 3880632 A			03-06-1993
		DE 3880632 T			18-11-1993
		EP 0318256 A			31-05-1989
		JP 1266858 A			24-10-1989
		JP 1854459 C			07-07-1994
		JP 5069585 B			01-10-1993
		JP 1168270 A			03-07-1989
		JP 7038788 B			01-05-1995
US 6087182	A 11-07-2000	WO 0013002 A			09-03-2000
US 5856174	A 05-01-1999	AU 6404996 A			05-02-1997
		EP 0843734 A			27-05-1998
		JP 11509094 T			17-08-1999
		WO 9702357 A			23-01-1997
		US 6043080 A			28-03-2000
		US 5922591 A			13-07-1999
WO 9641864	A 27-12-1996	NONE			
US 5567617	A 22-10-1996	AT 193465 T			15-06-2000
		DE 69517209 D			06-07-2000
		DE 69517209 T			23-11-2000
		EP 0662345 A			12-07-1995
		JP 7231798 A			05-09-1995
EP 0580362	A 26-01-1994	JP 6034546 A			08-02-1994
		US 6144448 A			07-11-2000

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 5213/00/WO-Por	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/08587	International filing date (day/month/year) 02/09/2000	Priority date (day/month/year) 04/09/1999	
International Patent Classification (IPC) or national classification and IPC B01L7/00			
Applicant F.HOFFMANN-LA ROCHE & CO. AG et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 22/12/2000	Date of completion of this report 18.12.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Marti, P Telephone No. +49 89 2399 7858



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/08587

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-14 as originally filed

Claims, No.:

1-24 with telefax of 03/12/2001

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
 - the language of publication of the international application (under Rule 48.3(b)).
 - the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

- 4. The amendments have resulted in the cancellation of:**

- the description, pages:
 - the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/08587

the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-24
	No:	Claims
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-24
Industrial applicability (IA)	Yes:	Claims 1-24
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Independent claims 1 and 13 have been amended by incorporating the features of dependent claims 11 and 22, respectively. Document WO-A-9838487 (D1) is still regarded as the most relevant state of the art. The system disclosed in claim 1 of the present application differs from the assembly unit described in D1 in that the cartridge has an outlet connected to the inlet by a channel, said channel having a protrusion such that the channel is longer than the shortest distance between inlet and outlet. However, the insertion of a protrusion in the channel to separate inlet from outlet is a constructional measure that a person skilled in the art would obviously consider in order to avoid that liquid exits directly the cartridge without filling the channel completely, when filled horizontally. Further, it can be seen in document EP-A-0318255 (D2), Figs. 2 and 3, that inlet and outlet are separated by a projection (38, side wall) such that the channel is longer than the shortest distance between inlet and outlet.

Therefore, the subject-matter of claim 1 does not involve an inventive step in the light of the disclosures of D1 and D2 (Art. 33.3 PCT).

The same applies to the subject-matter of claim 13.

2. Dependent claims 2-12 and 14-22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:
 - 2.1 Claims 2, 3, 14, 18 and 19. Document D1 describes a method of fabricating a chamber (cartridge) where the minor faces (light transparent walls) and the frame are moulded to a body having a reservoir with open sides. The major faces (heat conducting walls) are produced by attaching a film or sheet to the body (see p. 20).
 - 2.2 Claim 4. The optical system described in D1 can be used for carrying out fluorescence measurements (see p. 17).

- 2.3 Claim 5. Figure 6 in D1 shows heating/cooling plates which are in contact with the heating wall of the cartridge.
 - 2.4 Claims 6, 7 and 16. The conformable walls of the chamber in D1 mould to the angled shape of the heating surface (see p. 13 and Fig. 2b).
 - 2.5 Claim 8 and 17. The light transparent wall of the reaction chamber in D1 (see p. 17 and Fig. 5) has two sections: a first section through which the light enters the chamber and a second section through which the light exits the chamber.
 - 2.6 Claim 9. The feature of this claim is matter of normal design procedure, see also D1 (Fig. 5).
 - 2.7 Claim 10. Document D1 describes that the reaction chamber is inserted in the opening between two angled heating/cooling plates, which are made to bias against the chamber walls by springs (see p. 13 and Fig. 5).
 - 2.8 Claims 11, 12, 20, 21 and 22. Document US-A-5856174 (D3) discloses a miniaturized integrated DNA diagnostic device. It comprises reaction chambers which are from about 0.5 to about 20 mm in width or diameter and about 0.05 to about 5 mm deep.
 - 2.9 Claim 15. As mentioned in D1 (p. 14), the thickness of the wall in contact with the heating element should be between about 0.0001 to 0.02 inch (2.5 - 500 µm).
3. The method disclosed in claims 23 and 24 seems to describe nothing else than how does the system in claim 1 work, said system being not inventive. Thus, the subject-matter of claims 23 and 24 is not inventive (Art. 33.3 PCT).

Re Item VIII

Certain observations on the international application

1. Independent claim 1 contains all features of independent claim 13. In order to avoid lack of conciseness (Art. 6 PCT), claim 1 should be redrafted as a dependent claim on the present claim 13.
2. The channel width (lateral diameter) D_L is not clearly defined neither in claim 11 nor in the description (Art. 6 PCT). According to Fig. 1, D_L appears to include also part of the length of the protrusion.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/08587

The description should be brought in conformity with any new set of claims as required by Rule 5.1 (a)(iii) PCT and at least documents D1 and D2 reflecting the closest state of the art should be acknowledged in the description (R. 5.1 (a)(ii) PCT).

Claims

- 1 System for thermocycling of fluids in cartridges comprising
 - 5 a) a cartridge with at least one heat conducting wall and at least one light transparent wall, the cartridge having a fluid inlet and an outlet which are connected by a channel allowing flow-through filling of the cartridge
 - 10 b) a thermocycling unit in thermal contact with said heat conducting wall of said cartridge (1),
 - c) a light source (30) for transmitting light into the interior of said cartridge through said light transparent wall of said cartridge which is arranged substantially perpendicular to said heat conducting wall,
 - 15 d) a light detector (20) for detecting light emerging from the interior of the cartridge through said second wall,
 - e) a fluid providing unit coupled to an inlet of the cartridge for providing the cartridge with fluid by flow-through-filling.
- 20 2. System according to claim 1, wherein the cartridge has a body comprising the light transparent wall and having at least one opening which is sealed by a foil providing said heat conducting wall.
- 25 3. System according to claim 2, wherein the body is a frame which is sealed by two foils providing heat conducting walls.
4. System according to claim 1 for conducting fluorescent measurements wherein the light detector detects fluorescent light emerging from the cartridge.
- 30 5. System according to claim 1, wherein the thermocycling unit comprises at least one plate (40) in thermal contact with the heat conducting wall of the cartridge.

6. System according to claim 1, wherein the cartridge is wedge shaped and the thermocycling unit comprising a wedge shaped receiving section for receiving said wedge shaped cartridge.
- 5 7. System according to claim 6, where the opposing walls forming the wedge shaped cartridge comprise opposing walls having an angle of 3 to 8° one to another.
8. System according to claim 1, wherein said light transparent wall comprises a first section through which light is passed from the light source into the cartridge and a second section through which light is passed from the interior of the cartridge onto the light detector.
- 10 9. System according to claim 8, wherein said first section is tilted with respect to the illumination beam axis so that the light is refracted towards the second section of said transparent wall.
- 15 10. System according to claim 1 or 5, wherein said thermocycling unit comprises a plate (40) in thermal contact with said heat conducting wall of the cartridge and which exerts pressure onto said wall.
- 20 11. System according to claim 1, wherein said channel of said cartridge is longer than the shortest geometric distance D of inlet and outlet.
12. System according to claim 1, wherein said channel has a width D_L (lateral diameter) and a depth D_V (vertical diameter) and wherein the quotient of maximal width $D_{L,\max}$ and D_V is in the range of 1 to 10.
- 25 13. System according to claim 1 or 12, wherein the depth D_V (vertical diameter) of the channel is in the range of 0.5 to 5 mm.

14. Cartridge for conducting thermal cycling of fluids, comprising

- a) a substantially planar and heat conducting wall,
- 5 b) a light transparent wall which is disposed substantially vertical to said heat conducting wall,
- c) an inlet for providing the cartridge with fluid
- d) an outlet for draining fluid or gas from the cartridge.
- 10 e) a channel connecting inlet and outlet wherein said channel is adapted to allow flow-through-filling of the cartridge to avoid bubbles in a measuring section of the cartridge.

15. Cartridge according to claim 14, having two opposing heat conducting walls.

15 16. Cartridge according to claim 14 or 15, wherein the heat conducting wall is a foil with a thickness of less than 200 μm .

17. Cartridge according to claim 14 or 15, having two opposing walls from which at least one is a heat conducting wall and which form an angle of 3 to 8° with respect 20 to each other.

18. Cartridge according to claim 14, wherein the light transparent wall comprises a first section for transmitting light into the cartridge and a second section for transmitting light emerging from the cartridge.

25 19. Cartridge according to claim 14, which is made from a body having at least one opening which is closed by a heat conducting foil.

20. Cartridge according to claim 14, which is made from a frame which is closed by 30 two opposing foils.

21. Cartridge according to claim 14 having a thickness of 0,5 to 5 mm.

22. Cartridge according to claim 14, wherein said channel having a length which is longer than the shortest geometric distance D of inlet and outlet.
 - 5 23. Cartridge according to claim 14, wherein said channel has a width D_L (lateral diameter) and a depth D_V (vertical diameter) and wherein the quotient of maximal width $D_{L, \max}$ and D_V is in the range of 1 to 10.
 - 10 24. Cartridge according to claim 23, wherein the depth D_V (vertical diameter) of the channel is in the range of 0.5 to 5 mm.
 25. Method for thermocycling of fluids employing a system according to claim 1, with the steps
- 15
- filling the cartridge by a flow-through-process avoiding bubbles in a measuring section of the cartridge,
 - thermal cycling of fluid in the cartridge
 - transmitting light into the cartridge
 - detecting light emerging the cartridge.
- 20
26. Method according to claim 25 additionally monitoring the light emerging the cartridge to monitor amplification of nucleic acids in the fluid during thermal cycling.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17683 A2

(51) International Patent Classification⁷: B01L 7/00

CH-8932 Mettmenstetten (CH). CARATSCH, Thomas [CH/CH]; Im Gjuch 22, CH-8932 Mettmenstetten (CH). BIRRER, Lukas [CH/CH]; Baechtenbuehlstrasse 15, CH-6045 Meggen (CH). SAROFIM, Emad [CH/CH]; Flurstrasse 8, CH-6332 Hagendorn (CH).

(21) International Application Number: PCT/EP00/08587

(74) Common Representative: F. HOFFMANN-LA ROCHE & CO. AKTIENGESELLSCHAFT; c/o Roche Diagnostics GmbH, Patent Department, 68298 Mannheim (DE).

(22) International Filing Date:
2 September 2000 (02.09.2000)

(81) Designated States (national): JP, US.

(25) Filing Language: English

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

(26) Publication Language: English

Published:

— Without international search report and to be republished upon receipt of that report.

(30) Priority Data:
99117506.8 4 September 1999 (04.09.1999) EP

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(71) Applicant (for all designated States except US): F. HOFFMANN-LA ROCHE & CO. AKTIENGESELLSCHAFT [CH/CH]; Postfach 3255, CH-4002 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SCHNELL, Urban [CH/CH]; Lerchenweg 5, CH-6343 Rotkreuz (CH). ELSENHANS, Olivier [CH/CH]; Puentenstrasse 24,



WO 01/17683 A2

(54) Title: SYSTEM FOR THERMOCYLING OF FLUIDS IN CARTRIDGES

(57) Abstract: System for thermocycling of fluids in cartridges comprising a thermocycling unit in thermal contact with a first substantially planar and heat conducting wall of a cartridge, a light source for transmitting light into the interior of said cartridge through a second light transparent wall of said cartridge which is arranged substantially perpendicular to said first wall, a light detector for detecting light emerging from the interior of the cartridge through said second wall and a fluid providing unit coupled to an inlet of the cartridge for providing the cartridge with fluid and an outlet of the cartridge to drain gas when the cartridge is filled with fluid. The invention further concerns a cartridge for conducting the thermal cycling of fluids.

5 System for thermocycling of fluids in cartridges

- This invention is directed to a system for thermocycling fluids in cartridges to achieve an amplification and detection of nucleic acid sequences. A specific aspect of the present invention is that amplification and monitoring of the amplification process can be made
- 10 simultaneously without changing the position of the cartridge. A further aspect of this invention is the complete and bubble free filling of cartridges with fluids for thermocycling. The monitoring of the amplification process may be used to quantify the starting concentration of a target nucleic acid.
- 15 An apparatus for performing nucleic acid amplifications in reaction cartridges is known from US 5,567,617. This U.S. patent is concerned with an invention for nucleic acid amplification in a flexible cuvette. For amplification this cuvette is placed within a heater having a thin heating element with a central window providing an optical passage. Such an embodiment has the disadvantage that heating and detection compete for free space in the
- 20 lateral direction of the reaction cuvette. A further problem of the system used in US 5,567,617 is the flexible nature of the cuvette with fluid channels therein. Measures have to be taken to ensure that fluid communication within the cuvette is not obstructed when the cuvette is placed within the heater.
- 25 The present invention is directed to a system for thermocycling similar to the system described in US 5,567,617 but having the advantage that heating efficiency is not limited by free space needed for detection. A further advantage compared to US 5,567,617 is that a constriction of fluid passways within the cartridge is uncritical.
- 30 Document WO 98/38487 describes an assembly with a thermal sleeve for controlling the temperature within a fluid chamber. The assembly further comprises an optics for interrogation of the chamber content while the chamber is placed in the thermal sleeve.

The chamber disclosed however is designed to receive fluid by bottom up filling via a fluid port at the upper end of the chamber. Filling of such a chamber is uncritical if the inner diameter is large enough to allow fluid to enter without enclosure of air bubbles. Reduction of the diameter, however, is desirable to reduce for the internal distance between
5 temperature controlled walls to allow for sufficient heating and cooling rates of the fluid.

The aim of the present invention is to provide a system for rapid thermocycling of fluids in cartridges with on-line optical monitoring. A particular goal is to achieve rapid heating and cooling rates while avoiding the disturbance of optical measurements by bubbles enclosed
10 in the fluid.

This goal is achieved by a system with a thermocycling unit, a light source, a light detector, a fluid providing unit, and a cartridge in which thermocycling as well as detection can be conducted while the cartridge remains unchanged in position. Such a system allows
15 thermocycling with on-line optical monitoring. On-line monitoring means optical monitoring while temperature cycling is being performed. Furthermore the cartridge has a sufficiently small inner diameter between the temperature controlled walls to allow close temperature control of fluid in the cartridge . Filling of the cartridge is advantageously achieved by a flow-through process.

20 The cartridge of the present invention has the shape of a thin plate. The two opposing larger walls are used for heat transfer and one or more walls substantially vertical thereto are used for transmitting light into the cartridge and for transmitting light to be detected out of the cartridge. This cartridge design ensures that the optical path and the path for heat
25 exchange are spatially separated so that detection and heat transfer do not compete for space. The thickness of the cartridge is preferably in the range of 2.5 – 5mm and the area of each wall for heat transfer is preferably in the range of 0,1 – 4 cm². Such a cartridge can be made from a body having a bottom wall and upstanding side walls and the body being closed by a top wall to form a close cartridge with a reaction chamber therein. A preferable
30 embodiment of the cartridge is however made from a frame which provides the side walls of the cartridge and which is closed by a bottom and a top wall. It is particular preferred when top and bottom walls are foils with a thickness of less than 200µm. A cartridge in

accordance with the present invention may have a uniform thickness but it is however preferred when the heat conducting top and bottom walls form an angle of 3 to 8 degree with respect to each other. Such a wedge shaped cartridge has the advantage that it provides efficient heat transfer and easy handling when used in connection with a
5 thermocycling unit having a receiving section of a respective wedge shape.

Cartridges of the present invention can be made from transparent plastics. Preferably an annular shaped frame with a wall thickness of 0,3 – 4 mm is provided to form the side walls of the cartridge. The frame can be made e.g. by injection molding from polymers as
10 polyethylen or polypropylen. The frame is closed by foils forming top and bottom walls by heat sealing. Processes for sealing a polymer foil onto such a frame are well known in the art and will therefore not be discussed in detail herein. Heat sealing processes are e.g. described in: Polymere Werkstoffe: 3rd volume, Editor: Hans Batzer.

Stuttgart;New York: Thieme. Bd. 2. Technologie 1, 1984, pages 206-212;

15 Kunststoffverarbeitung; Editor: Otto Schwarz et al.; 4th revised edition Würzburg: Vogel, 1988, pages 193 – 206 and Kunststoff-Folien: Herstellung, Eigenschaften, Anwendung; Editor: Joachim Nentwig. München, Wien: Hanser, 1994, pages 88 – 93. Metallised multilayer foils can also be employed for sealing onto the frame.

20 As will become clearer below in connection with the filling process the cartridge is being made from materials with high surface tension or the surface tension of inner walls can be enhanced. Such an enhancement of surface tension can be achieved by adequate oxid coating or plasma treatment of the surface. The limiting surface tension value enabling bubble free filling, however, depends on the surface tension of the fluid.

25

As mentioned it is advantageous to employ cartridges with a small internal diameter between the heat controlled walls of the cartridge. It has shown that a diameter below approx. 5 mm allows close temperature control and thus high heating and cooling rates.

30 It should be noted that the interior of the cartridge may have fluid channels, as well as protrusions and recesses. Furthermore the used language "closed cartridge" does not

exclude fluid channels intersecting the walls of the cartridge which are used for bringing fluids into or out of the cartridge.

The thermocycling unit of the present invention comprises a heating section for establishing a thermal contact with the heat conducting walls of the cartridge. The heating section preferably comprises at least one plate which is brought into mechanical contact with the heat conducting wall of the cartridge and the plate itself being heated and cooled as necessary for the thermocycling process. This heating and cooling can be made by e.g. Peltier elements, bringing the plate into contact with hot and cold fluids or by heating with a resistor heater and cooling by blowing air onto the plate. Procedures and apparatuses for performing thermocycling by thermal contact with plates are well known in the art.

It should be understood, however, that other devices for heating and cooling the cartridges can be used without departing from the scope of the invention. It is only necessary that whatever device is used for heating and cooling the cartridges, be capable of reaching and sustaining the temperatures involved and achieve the desired temperature versus time profile. Thus, for purposes of nucleic acid amplification, such a device should be capable of cycling the temperature of the amplification reaction mixture between a denaturing temperature T_1 (which can be in the range of about 80-105°C and preferably 90-100°C) and an annealing/extension temperature T_2 (which can be in the range of about 30-90°C and preferably 50-70°C) where $T_1 > T_2$ as is known to those skilled in the art.

To achieve a sufficient thermal contact between the cartridge and the heating section means can be provided which press one or two plates against the cartridge. Such means are e. g. described in US 5,567,617. In the context of the present invention it is however preferred to employ a heating unit having a receiving section of a wedge shaped recess. The receiving section may be formed by two walls which are inclined one to the other. A wedge shaped cartridge can be simply placed into such a wedge shaped receiving section and sufficient thermal contact between the walls of the receiving section and the heat conducting walls of the cartridge is automatically achieved by mechanical contact into which the respective walls come when the cartridge runs down into the receiving section.

The thermocycling unit further comprises a steering unit which steers the timely heating and cooling of the heater section. Such a steering section is for example described in EP B 0 236 069 which is hereby incorporated by reference . The present invention does not need a steering unit different to the one described in EP B 0 236 069 but timing, temperature profiles and the amount of heat exchanged with the cartridge has to be adapted to the specific needs.

5 The present invention involves nucleic acid amplification and the detection, monitoring and quantification of amplification products. In order to facilitate understanding of the 10 amplification data collection and processing system of the present invention, a summary of nucleic acid amplification processes especially suited for use in conjunction with the invention will first be discussed.

15 Those of skill will recognize that the present invention requires amplification of the duplex form of nucleic acid. There exist well-known methods for amplifying nucleic acids. The means for amplification are not critical and this invention will work with any method where nucleic acid duplexes are generated. The various methods are reviewed in Bio/Technology 8:290-293, April 1990. They include, but are not limited to PCR, LCR, Q β and 3SR. Although 3SR and Q β do not involve thermal cycling, the result of their 20 amplifications can be monitored by the fluorescence detecting arrangement discussed below and analyzed in accordance with the principles of the present invention. Each method is briefly described below.

25 PCR amplification of DNA involves repeated cycles of heat denaturing the DNA, annealing two oligonucleotide primers to sequences that flank the DNA segment to be amplified, and extending the annealed primers with DNA polymerase. The primers hybridize to opposite strands of the target sequence and are oriented so that DNA synthesis by the polymerase proceeds across the regions between the primers, each successive cycle essentially doubling the amount of DNA synthesized in the previous cycle. This results in 30 the exponential accumulation of the specific target fragment, at a rate of approximately 2^n per cycle, where n is the number of cycles. A complete review of this technology can be found in PCR Technology- Principles and Applications, Ed. Erlich H.A., Stockton Press,

Now York 1989. Taq DNA polymerase is preferred when PCR is used in conjunction with the present invention although this is not an essential aspect of the invention.

The ligase chain reaction is described in International Patent Application WO 89/09835.

5 The process involves the use of ligase to join oligonucleotide segments that anneal to the target nucleic acid. Ligase chain reaction (LCR) results in amplification of an original target molecule and can provide millions of copies of product DNA. Consequently, the LCR results in a net increase in double-stranded DNA. The present detection methods are applicable to LCR, as well as PCR. LCR typically requires some means for detecting the
10 product DNA such as an oligonucleotide probe. When used in conjunction with the disclosed methods for detecting amplification products, such means are unnecessary, and the LCR result is immediately detectable.

Another amplification scheme, Q-beta replicase, exploits the use of the replicase from the

15 RNA bacteriophage Q β . In this amplification scheme, a modified recombinant bacteriophage genome with a sequence specific for the targeted sequence is initially ligated to the nucleic acid to be tested. Following enrichment of the duplexes formed between the bacteriophage probe and the nucleic acid in a sample, Q β replicase is added, which, upon recognizing the retained recombinant genome, begins making a large number of copies.

20 The Q β system does not require primer sequences and there is no heat denaturation step as with the PCR and LCR amplification systems. The reaction occurs at one temperature, typically 37°C. The preferred template is a substrate for the Q β replicase, midvariant-1 RNA. A very large increase in the templates is achieved through the use of this system. A review of this amplification system can be found in International Patent Application WO
25 87/06270 and in Lizardi et al., 1988, Bio/Technology 6:1197-1202.

The 3SR system is a variation of an in vitro transcription-based amplification system. A

transcription-based amplification system (TAS) involves the use of primers that encode a promoter sequence as well as a complementary sequence to the target strand to generate

30 DNA copies of a target strand and the production of RNA copies from the DNA copies with an RNA polymerase. See, e.g., Example 9B of U.S. Patent No. 4,683,202 and

European Patent Application EP-A-0 310,229. The 3SR System is a system which uses three enzymes to carry out an isothermal replication of target nucleic acids.

The system begins with a target of singlestranded RNA to which a T7 RNA DNA primer is bound. By extension of the primer with reverse transcriptase, a cDNA is formed, and RNaseH treatment frees thecDNA from the heteroduplex. A second primer is bound to the cDNA and a doublestranded cDNA is formed by reverse transcriptase treatment. One (or both) of the primers encodes a promoter, e.g., the promoter for T7 RNA polymerase, so that the double-stranded cDNA is a transcription template for RNA polymerase.

10

Transcription competent cDNAs yield antisense RNA copies of the original target. The transcripts are then converted by the reverse transcriptase to double-stranded cDNA containing double-stranded promoters, optionally on both ends in an inverted repeat orientation. These DNAs can yield RNAs, which can reenter the cycle. A more complete description of the 3SR system can be found in Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878, and European Patent Application EP-A-0 329 822.

According to the present invention, nucleic acid amplification is monitored by detecting fluorescence emitted when a fluorescent dye such as an intercalating fluorescent dye,

20 provided in the reaction mixture, binds with the double-stranded nucleic acid during each annealing/extension phase as the mixture is cycled between two temperatures (thermal cycling). An increase in fluorescence indicates a positive amplification of target nucleic acid. Suitable intercalating agents or dyes include, but are not limited to ethidium bromide, propidium bromide, proflavine, acridine orange, acriflavine, fluorcoumarine, ellipticine, 25 daunomycin, chloroquine, distamycin D, chromomycin, homidium, mithramycin, ruthenium polypyridyls, anthramycin, methidium bromide, 2-[2-(4-hydroxyphenyl)-6-benzimidazole-6-(1-methyl4-piperazye) benzimidazole trihydrochloride and the like.

Fluorophores and DNA binding chromophores described in the art are suitable for use in the 30 5' to 3' nuclease assay disclosed in U.S. Patent No. 5,210,015 are also useful in the present invention. Suitable donor fluorophores and quenchers are chosen such that the emission spectrum of the donor fluorophore overlaps with the absorption spectrum of the

quencher. Ideally, the fluorophores should have a high Stokes shift (a large difference between the wavelength for maximum absorption and the wavelength for maximum emission) to minimize interference by scattered excitation light.

- 5 Suitable labels which are well known in the art include, but are not limited to, fluorescein and derivatives such as FAM, HEX, TET, and JOE; rhodamine and derivatives such as Texas Red, ROX, and TAMRA; Lucifer Yellow, and coumarin derivatives such as 7-Me2N-coumarin-4-acetate, 7OH-4-CH₃-coumarin-3-acetate, and 7-NH₂-4-CH₃coumarin-3-acetate (AMCA). FAM, HEX, TET, JOE, ROX , and TAMRA are marketed by Perkin
10 Elmer, Applied Biosystems Division (Foster City, CA). TEX-as Red and many other suitable compounds are marketed by Molecular Probes (Eugene, OR). Examples of chemiluminescent and bioluminescent compounds that may be suitable for use as the energy donor include luminol (aminophthal-hydrazide) and derivatives, and Luciferases.
- 15 The optics of a system in accordance with the present invention comprises a light source and a light detector . With the system absorption or scattering measurements with the fluid within the cartridge can be performed. It is however preferred to use this system for fluorescent measurements where light is transmitted into the interior of the cartridge to initiate fluorescence emission which is detected by the light detector. The light source may
20 comprise semi-conductor light sources as well as halogen lamps or other embodiments. Within the spirit of the present invention the light is transmitted into the cartridge through a second light transparent wall of the cartridge which is substantially perpendicular to the wall for heat transfer. Due to the flat shape of the cartridge the second light transparent wall has a width of only 0,5 to 5 mm in one dimension. It is therefor preferred to employ
25 beam shaping optics cooperating with the light source to introduce light through this restricted window. Such beam shaping optics may include apertures, lenses and fibre optics. For fluorescent measurements it is necessary to stimulate fluorescent dyes with light of a wavelength within the absorption spectrum of the dye. It is normally desired to suppress background radiation caused by light emission from other sources than the
30 fluorescent dye. In many embodiments it is furthermore desirable to perform fluorescent measurements with two or more dyes within the same reaction volume. In these cases it is necessary to restrict the bandwidth of the light for illumination. This can be done by using

light sources having a narrow bandwidth as for e.g. LED's or by using light sources having a broad bandwidth in connection with suitable filters.

- A system of the present invention further comprises one or more light detectors for
5 detecting light emerging from the interior of the cartridge through the second light transparent wall. Suitable light detectors are semiconductor detectors as photodiodes or photomultipliers. As already mentioned it is desirable to suppress background signals and therefore detection should be limited to the emission band of the fluorescent dye to be detected. This can be achieved by wavelength filters or by spectral separation of the light
10 with a spectrometer. Within the present invention it is preferred to detect two or more fluorescent dyes within the same cartridge by separate detection of the emitted fluorescent radiation from the dyes. Advantageously the dyes are also stimulated separately with suitable light sources.
- 15 The light detector of the present invention may cooperate with light directing means as fiberoptics, lens systems and so on.

- The cartridge of the present invention is used as a thermocycling cavity for fluids and it therefore has an inlet for providing the cartridge with fluid. Optionally the cartridge may
20 have an outlet for draining fluid from the cartridge. It is however preferred in some modes of use to have a disposable cartridge without an outlet for liquid. Such a cartridge can be discarded together with its content after use. The inlet and the optional outlet are preferably channels through the body or through the frame of the cartridge. As already mentioned body or frame are made mostly incompressible so that the fluid channels are not obstructed
25 when pressure is applied to the cartridge in order to achieve a thermal contact with the heating unit. A system in accordance with the present invention comprises a fluid providing unit which is coupled to the inlet of the cartridge. Such a fluid providing unit may be a pipetting unit or an internal fluid channel of the system from which fluid is pressed into the cartridge. The system optionally comprises a fluid receiving unit which is
30 coupled to an outlet of the cartridge to receive fluid from the cartridge. Such a fluid receiving unit is necessary if the cartridge has to be emptied or if different fluids have to be processed within the same cartridge. In preferred embodiments the cartridge is however

discarded together with the fluid therein after single use. Such embodiments do not need a fluid receiving unit but only an outlet to drain gas when the cartridge is filled with fluid.

Cartridges in accordance with the present invention are preferably filled by the so called flow-through-filling. Flow-through filling means that fluid is introduced via an inlet and fills the inner space of the cartridge directly adjacent the inlet. Introduction of further liquid continuously enlarges the filled space until a desired degree of filling is achieved. In this process the fluid bolus is never released from the inlet during filling as it is the case for bottom-to-top-filling as described in WO 98/38487. To allow for a flow-through-filling process the inner geometry of the cartridge, respectively the channel connecting inlet and outlet is important. For thermocycling cartridges it is desirable to have a large contact area of fluid with the thermally controlled walls. The dimension of the channel which controls the contact area will be denoted D_L (lateral diameter) and the internal distance between the thermally controlled walls D_V (vertical diameter). The larger the quotient D_L/D_V the more efficient is heat control as well as the risk that bubbles are enclosed during filling. It has shown that optimal quotients D_L/D_V are in the range of 1 to 10.

Bearing in mind that the risk of bubble formation increases with increasing lateral diameter the quotient should be calculated employing the largest vertical diameter (D_V, \max) of the channel, wherein the diameter is measured vertical to the flow direction.

Other factors as surface tension (of wall and fluid), viscosity of the fluid, capillary forces and filling rate also will influence the filling process. Said fluid channel, however does not necessarily have a unique shape at each section. It has shown advantageous to employ a curved channel so that the length L of the channel is larger than the geometric distance D between inlet and outlet. Such a design can efficiently be generated by a protrusion projecting into the interior of the cartridge as shown in figure 1.

The cartridge may also have a space for receiving gases which are set free during heating of the fluid. This space is arranged in a section of the cartridge which does not interfere with optical measurement.

The present invention is further described in more detail with the regard to the following figures:

5 Figure 1: Cartridge in top view partially filled with fluid

Figure 2: Cartridge in top view with light sources and detector

10 Figure 3: Light source module

15 Figure 4: Raytrace diagram with cartridge having an oblique window

Figure 5: Fluorescence signal during thermocycling

20 Figure 6: System for thermocycling in a cartridge within a metal block thermocycler, integrating light sources and light detector.

Figure 7: System for thermocycling with wedge shaped cartridge

25 Figure 8: Filling process of cartridge shown in figure 1.

Figure 1 shows a cartridge (1) for thermocycling of fluids in top view. The cartridge has a body (3) with a cavity (2) therein. The cartridge body has been molten from a solid polypropylene block. In the region of the cavity material was removed until a cell bottom of 200 μ m thickness had been achieved. (In serial production the cartridge can be produced more efficiently by injection molding.) Into the polypropylene block has also been molten an inlet (10) to receive fluid and an outlet (11) to vent air from the cavity during filling. The body of the cartridge shown in figure 1 has been closed by welding a sealing foil of 40 μ m thickness onto the cartridge body. Figure 1 shows a preferred shape of the cavity (2) with a protrusion (5) in form of a nose reaching into the cavity. The protrusion (5) ensures that the cavity can be filled completely with fluid without enclosure of air bubbles.

The protrusion modifies the geometry of the cartridge so that the largest lateral diameter $D_{L, \text{max}}$ is reduced as well as the quotient $D_{L, \text{max}}/D_v$ (D_v is the distance between the heat controlled walls; i.e. the thickness of the fluid in the cartridge). In the shown experimental setup $D_{L, \text{max}}$ was 4.0 mm and D_v was 1.5 mm resulting in a quotient of 2.7.

5

The size of protrusion (5) has to be adapted to this specific situation and depends on cavity thickness, viscosity of the liquid, surface tension of bottom and top wall of the cartridge as well as size and shape of the cavity. It has been found that the effect of the protrusion is to avoid a fluidic shortcut between inlet and outlet. In this spirit the protrusion directs the

10 fluid on a path (L) which is longer than the shortest distance (D) between inlet and outlet.

In Figure 1 there have been shown several fluid paths by dotted lines. It has been found to be advantageous if the quotient of the shortest L divided by D is two or more. Figure 1 furthermore shows a preferred embodiment with respect to the location of inlet and outlet on the same side of the cartridge. With this arrangement it is possible to locate fluid

15 connections on the same side and make connection to an apparatus easier. A disposal of inlet and outlet on the same side is also advantageous in that this side may be located

upside within the system during filling, cycling and measurement. By this arrangement air bubbles remaining occasionally in the cavity or being created during heating of the cartridge can be eliminated through the inlet or the outlet port. Figure 1 also shows an

20 advantageous shape of the cavity adjacent inlet and outlet. As can be seen the walls, (7, 7', 7'', 7''') form an angle with the inlet and outlet of approximately 130°. A preferred range of this angle is 100 - 150° which has shown to avoid empty spots when filling the cavity with fluid. A further aspect of the present invention which can be seen in figure 1 are the oblique windows (4, 4'). Light is irradiated into the cavity perpendicular to surfaces (8, 8')

25 of the cavity and is detected by schematically shown detector (20). The irradiating light is being refracted by the oblique surfaces (4, 4') in a direction towards the detector. Due to this arrangement the generation of fluorescent light can be directed to a central portion of the cavity which is closer to the detector in order to increase the measurement sensitivity.

30 Figure 2 shows a schematic drawing of a cartridge in combination with light sources (30, 30', 30'', 30''') and a detector (20). As can be seen the focal point of the light sources is being disposed in the centre of the cavity and the focal points are mostly coincident. The

detector (20) is disposed beneath the detection window of the cartridge to detect fluorescent light from the centre of the cavity. With the setup of figure 2 it is possible to monitor the fluorescence of four different fluorescent dyes. Each of the light sources is chosen to specifically stimulate one of the fluorescent dyes. By subsequent activation of 5 the light sources the amount of each fluorescent dye can be monitored by detection of fluorescent radiation with the detector.

Figure 3 shows a light source module which can be advantageously used within the present invention. The light source module comprises a semiconductor light source (31) a 10 wavelength selection filter (32) and an optical output power monitor (33). The output power monitor comprises a beam splitter (33a) to extract a portion of the light from the light path and a detector (33b) to detect the extracted light. The amount of light detected is used to steer the current applied to the semiconductor light source so as to generate a constant and specific output. The light source module of figure 3 further comprises a beam 15 shaper (34) in form of a lens. Advantageously the light emitting surface of the semiconductor light source is imaged by the lens into the centre of the cartridge.

Figure 4 shows a raytrace of a system employing a cartridge with an oblique window. As 20 can be seen the light generated by light source (30) is imaged by a ballshape lens (35) onto an oblique window (4) of the cartridge. Due to this arrangement the light is refracted in direction of the detector (20).

Figure 5 depicts a fluorescence over time diagram which was measured with the system according to the present invention. The abscissa shows the number of the measurement and 25 the ordinate shows fluorescence intensity in arbitrary units. Measurements were taken after each denaturation, elongation cycle of the polymerase chain reaction. A cartridge (1) as shown in figures 1 and 6 has been introduced and fixed in a holder as shown in figure 6. The holder with the cartridge had been integrated into a thermocycler unit. Illumination optics with simple beam shaping optics and detection optics have been used for 30 quantitative fluorescence measurement. PCR runs (HCV-control with Mastermix, 5×10^3 initial copies) with the following protocol have then been performed: 120 cycles, denaturation temperature $T_{\text{denat}} = 94^\circ\text{C}$, annealing temperature $T_{\text{anneal}} = 60^\circ\text{C}$, tempearture

ramp time t_{ramp} approx. 20 s, temperature plateau $t_{plateau}$ approx. 26 s, total process time t_{tot} approx. 3 h. The experimentally measured fluorescence signal vs. number of measurement is plotted in Fig. 5 (solid line). For comparison, the fluorescence signal of an amplification within a vessel has been measured with the same PCR protocol. The result is also shown in Fig. 5 (dotted line). The decreased fluorescence signal of the cartridge (approx. factor 2) is a result of the beam shaping optics used for measurement. This optics introduces an illumination power loss of approximately 30 %.

Figure 6 shows a cartridge of the present invention in the cavity of a metal block for thermocycling. Within the metal block there are integrated light sources and a light detector. For thermocycling the closure (41) can be screwed onto the metal block (40) so as to achieve an optimum thermal contact between the cartridge and the metal block. The metal block itself is provided with Peltier elements for heating and cooling.

Figure 7 shows an advantageous system in accordance with the present invention. The cartridge shown is a wedge shaped embodiment having a larger thickness on the side of inlet and outlet. The system is provided with a wedge shaped receiving unit comprising of Peltier elements (40') forming an angle one to the other so as to receive the wedge shaped cartridge. With this embodiment it is possible to achieve an intensive thermal contact between the cartridge and the heating unit by simply inserting the cartridge between the Peltier elements (40'). This figure shows that the optical path and the path for heat exchange are spatially separated so that detection and heat transfer do not compete for space.

Figure 8 shows the filling of the cartridge of figure 1 with fluid. Fluid is introduced through inlet (10) and fills the cartridge region directly adjacent the inlet (picture a)). Further introduction of fluid enlarges the filled region as can be seen in figures 8b) and c). This flow-through-filling process provides a bubble free filling of the cartridge.

Claims

- 1 System for thermocycling of fluids in cartridges comprising
 - 5 a) a cartridge with at least one heat conducting wall and at least one light transparent wall, the cartridge having a fluid inlet and an outlet which are connected by a channel allowing flow-through filling of the cartridge
 - 10 b) a thermocycling unit in thermal contact with said heat conducting wall of said cartridge (1),
 - c) a light source (30) for transmitting light into the interior of said cartridge through said light transparent wall of said cartridge which is arranged substantially perpendicular to said heat conducting wall,
 - d) a light detector (20) for detecting light emerging from the interior of the cartridge through said second wall,
 - 15 e) a fluid providing unit coupled to an inlet of the cartridge for providing the cartridge with fluid by flow-through-filling.
- 20 2. System according to claim 1, wherein the cartridge has a body comprising the light transparent wall and having at least one opening which is sealed by a foil providing said heat conducting wall.
- 25 3. System according to claim 2, wherein the body is a frame which is sealed by two foils providing heat conducting walls.
4. System according to claim 1 for conducting fluorescent measurements wherein the light detector detects fluorescent light emerging from the cartridge.
- 30 5. System according to claim 1, wherein the thermocycling unit comprises at least one plate (40) in thermal contact with the heat conducting wall of the cartridge.

6. System according to claim 1, wherein the cartridge is wedge shaped and the thermocycling unit comprising a wedge shaped receiving section for receiving said wedge shaped cartridge.

5 7. System according to claim 6, where the opposing walls forming the wedge shaped cartridge comprise opposing walls having an angle of 3 to 8° one to another.

10 8. System according to claim 1, wherein said light transparent wall comprises a first section through which light is passed from the light source into the cartridge and a second section through which light is passed from the interior of the cartridge onto the light detector.

15 9. System according to claim 8, wherein said first section is tilted with respect to the illumination beam axis so that the light is refracted towards the second section of said transparent wall.

20 10. System according to claim 1 or 5, wherein said thermocycling unit comprises a plate (40) in thermal contact with said heat conducting wall of the cartridge and which exerts pressure onto said wall.

11. System according to claim 1, wherein said channel of said cartridge is longer than the shortest geometric distance D of inlet and outlet.

25 12. System according to claim 1, wherein said channel has a width D_L (lateral diameter) and a depth D_V (vertical diameter) and wherein the quotient of maximal width $D_{L_{\max}}$ and D_V is in the range of 1 to 10.

13. System according to claim 1 or 12, wherein the depth D_V (vertical diameter) of the channel is in the range of 0.5 to 5 mm.

14. Cartridge for conducting thermal cycling of fluids, comprising

- a) a substantially planar and heat conducting wall,
- 5 b) a light transparent wall which is disposed substantially vertical to said heat conducting wall,
- c) an inlet for providing the cartridge with fluid
- d) an outlet for draining fluid or gas from the cartridge.
- 10 e) a channel connecting inlet and outlet wherein said channel is adapted to allow flow-through-filling of the cartridge to avoid bubbles in a measuring section of the cartridge.

15. Cartridge according to claim 14, having two opposing heat conducting walls.

15 16. Cartridge according to claim 14 or 15, wherein the heat conducting wall is a foil with a thickness of less than 200 μ m.

17. Cartridge according to claim 14 or 15, having two opposing walls from which at least one is a heat conducting wall and which form an angle of 3 to 8° with respect
20 to each other.

18. Cartridge according to claim 14, wherein the light transparent wall comprises a first section for transmitting light into the cartridge and a second section for transmitting light emerging from the cartridge.

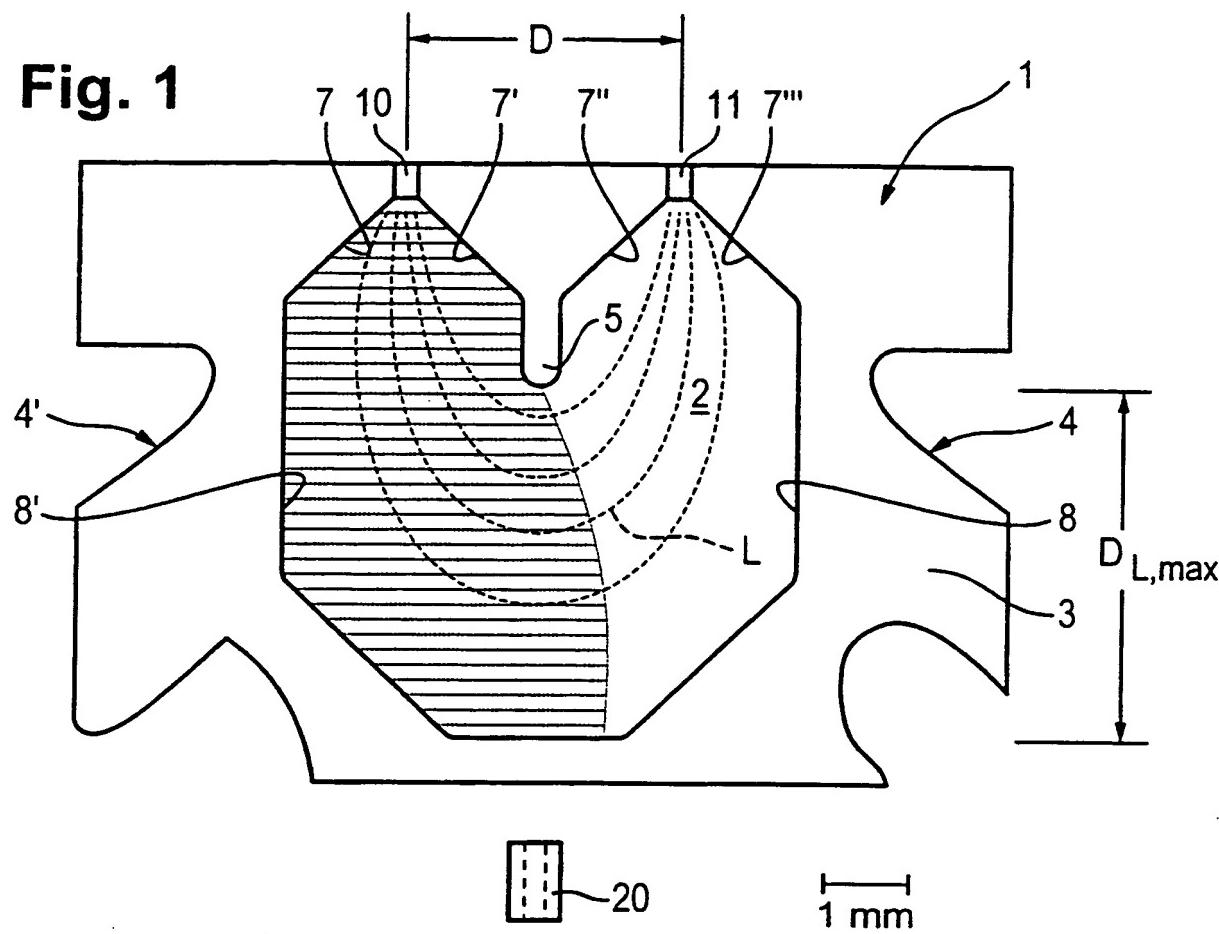
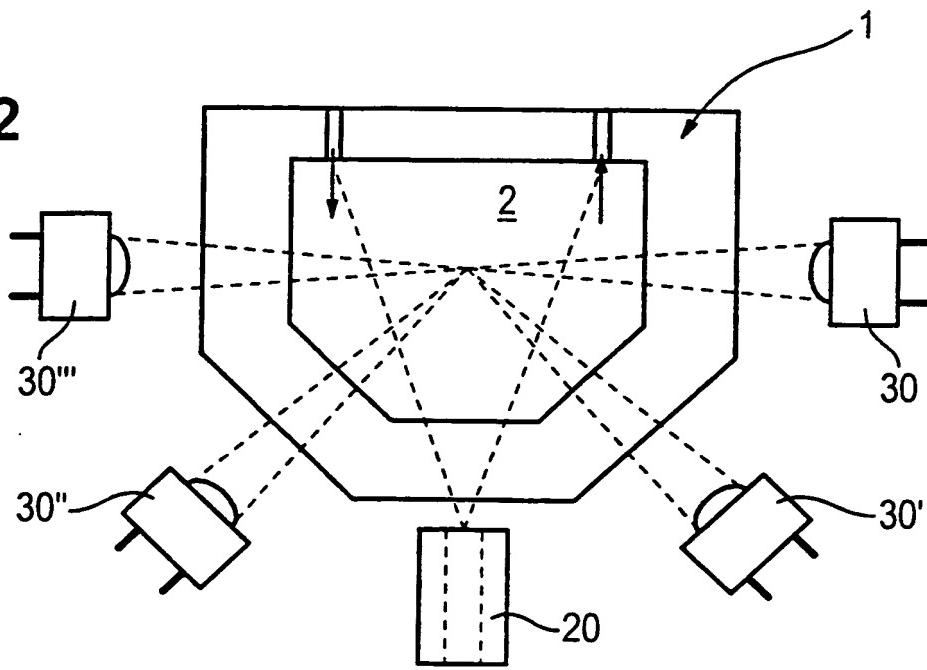
25 19. Cartridge according to claim 14, which is made from a body having at least one opening which is closed by a heat conducting foil.

20. Cartridge according to claim 14, which is made from a frame which is closed by
30 two opposing foils.

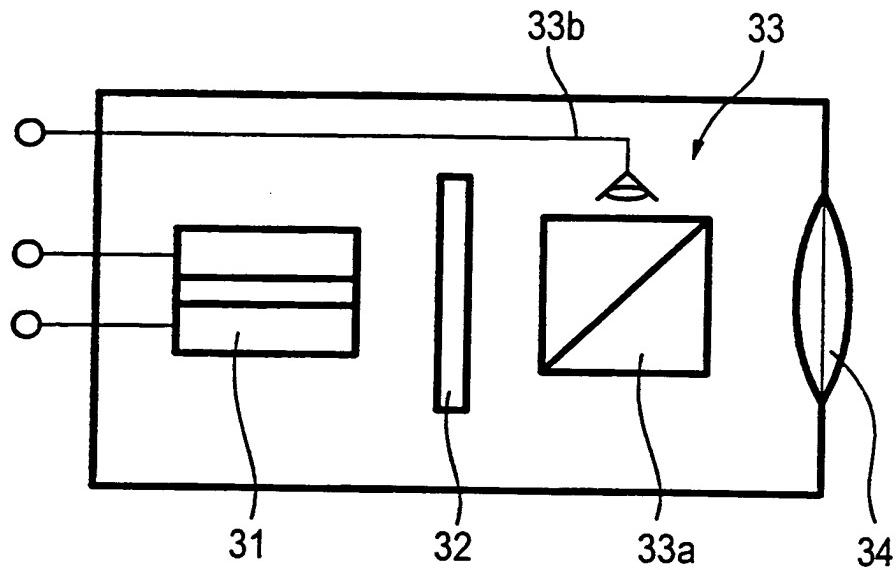
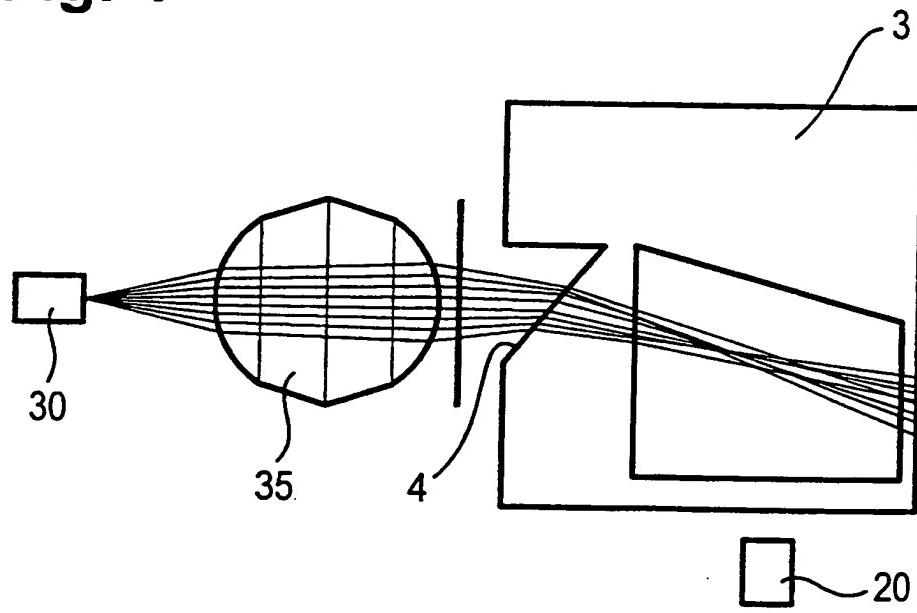
21. Cartridge according to claim 14 having a thickness of 0,5 to 5 mm.

22. Cartridge according to claim 14, wherein said channel having a length which is longer than the shortest geometric distance D of inlet and outlet.
- 5 23. Cartridge according to claim 14, wherein said channel has a width D_L (lateral diameter) and a depth D_V (vertical diameter) and wherein the quotient of maximal width $D_{L,\max}$ and D_V is in the range of 1 to 10.
- 10 24. Cartridge according to claim 23, wherein the depth D_V (vertical diameter) of the channel is in the range of 0.5 to 5 mm.
25. Method for thermocycling of fluids employing a system according to claim 1, with the steps
- 15 - filling the cartridge by a flow-through-process avoiding bubbles in a measuring section of the cartridge,
 - thermal cycling of fluid in the cartridge
 - transmitting light into the cartridge
 - detecting light emerging the cartridge.
- 20 26. Method according to claim 25 additionally monitoring the light emerging the cartridge to monitor amplification of nucleic acids in the fluid during thermal cycling.

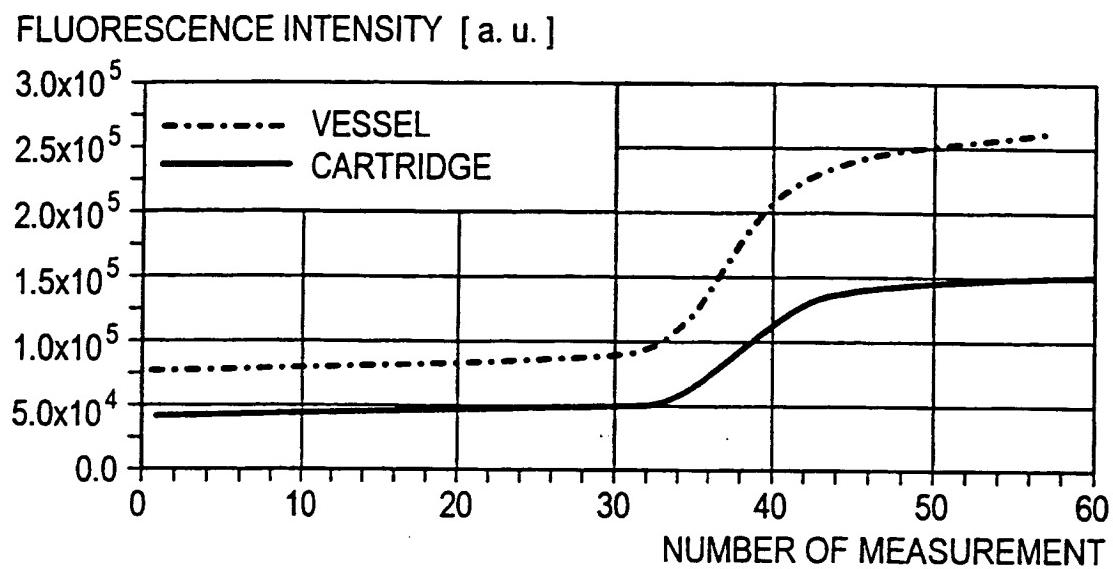
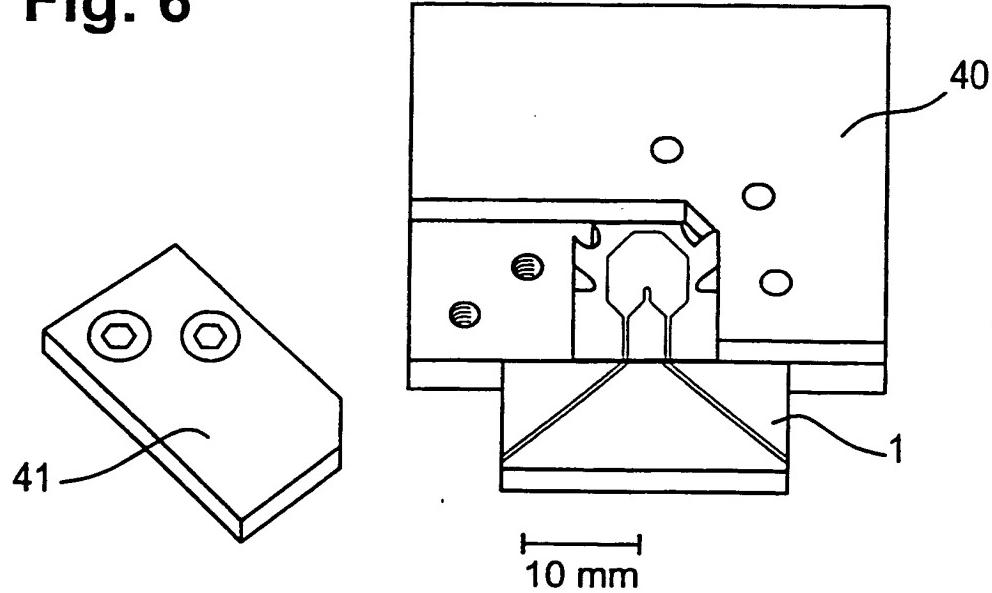
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Fig. 1**Fig. 2**

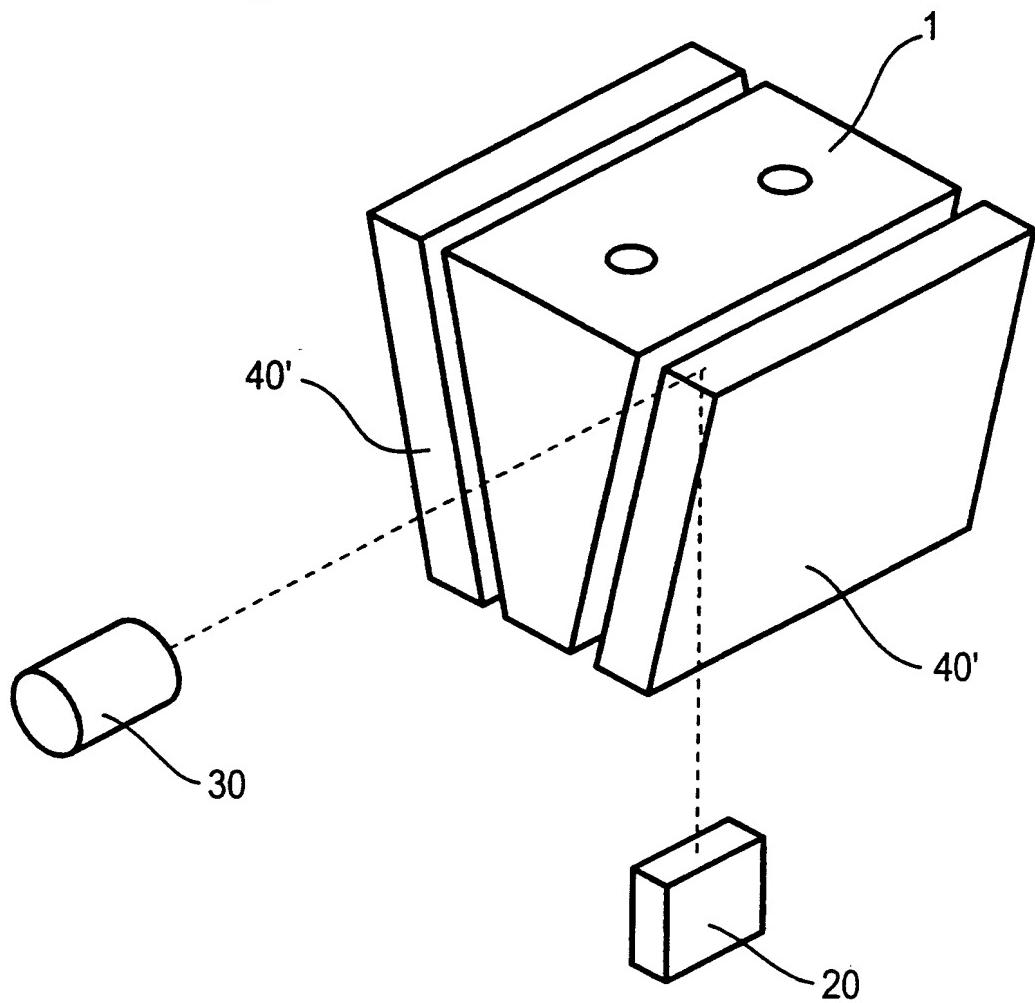
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Fig. 3**Fig. 4**

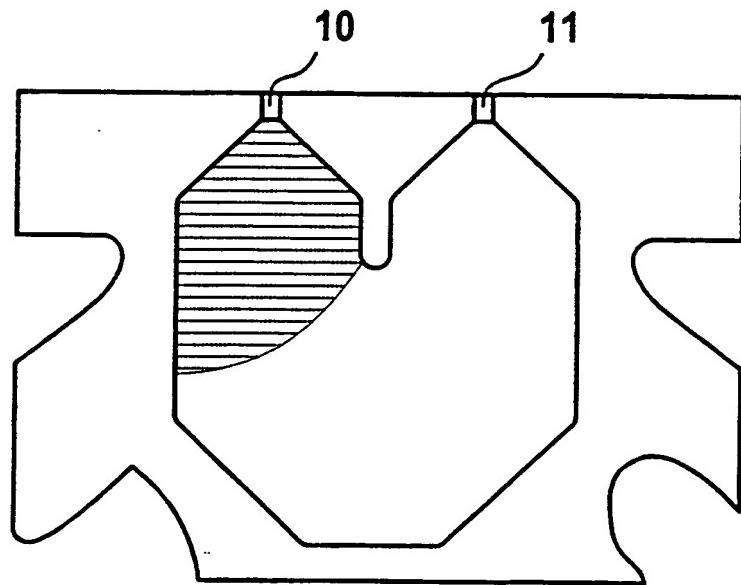
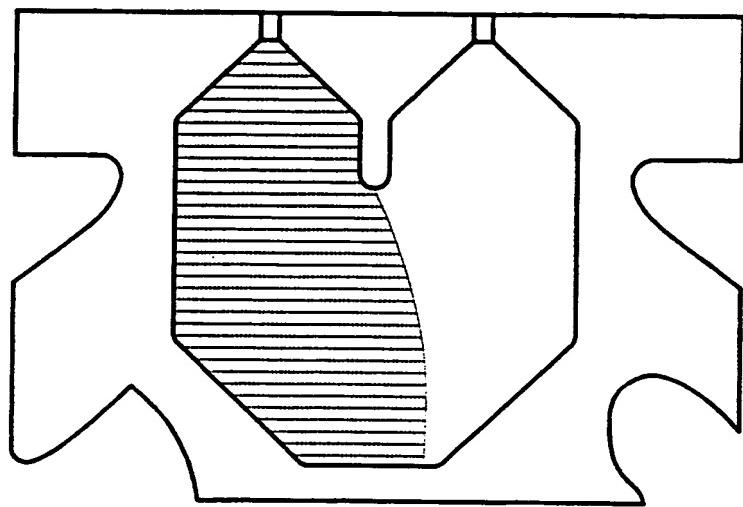
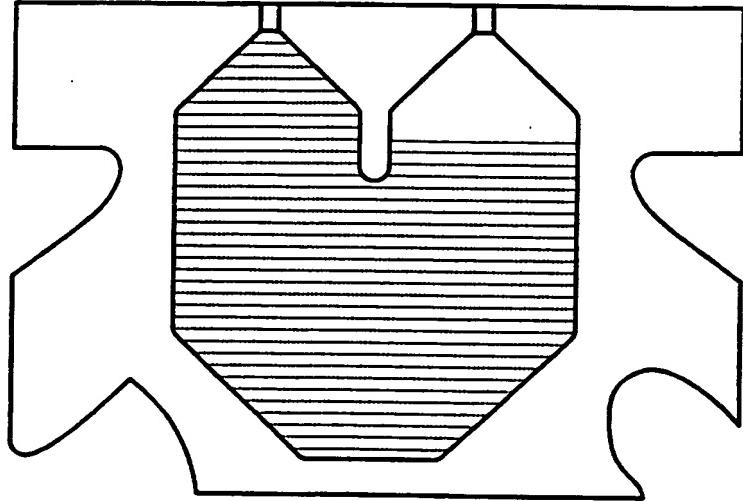
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Fig. 5**Fig. 6**

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Fig. 7

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Fig. 8**a)****b)****c)****SUBSTITUTE SHEET (RULE 26)**

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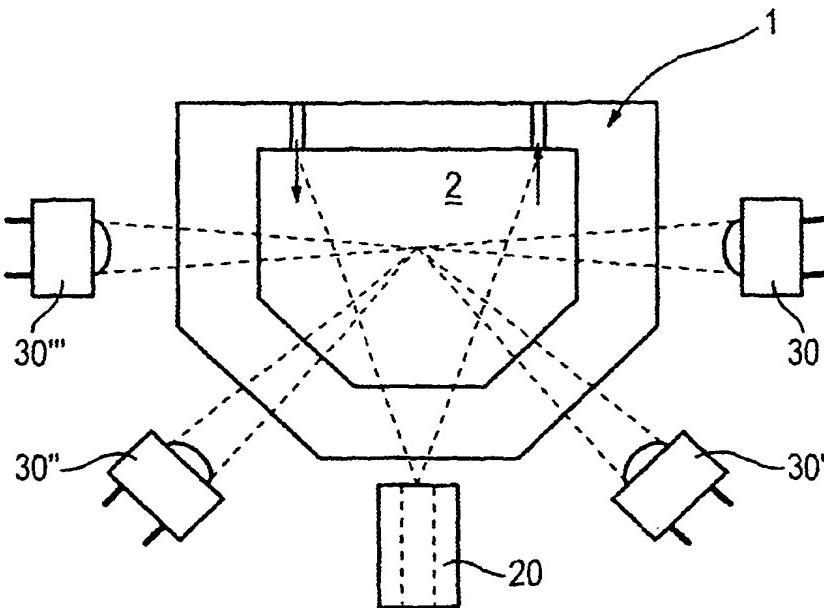
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[Continued on next page]

(54) Title: SYSTEM FOR THERMOCYCLING OF FLUIDS IN CARTRIDGES



WO 01/17683 A3

(57) Abstract: System for thermocycling of fluids in cartridges comprising a thermocycling unit in thermal contact with a first substantially planar and heat conducting wall of a cartridge, a light source for transmitting light into the interior of said cartridge through a second light transparent wall of said cartridge which is arranged substantially perpendicular to said first wall, a light detector for detecting light emerging from the interior of the cartridge through said second wall and a fluid providing unit coupled to an inlet of the cartridge for providing the cartridge with fluid and an outlet of the cartridge to drain gas when the cartridge is filled with fluid. The invention further concerns a cartridge for conducting the thermal cycling of fluids.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

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INTERNATIONAL SEARCH REPORT

Inter

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